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Metabolic Model for *Tetrasphaera* in Enhanced Biological Phosphorus Removal Plants

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INTRODUCTION

Enhanced biological phosphorus removal (EBPR) is an environmentally friendly and cost-effective phosphorus removal process for wastewater. It is believed to be carried out mainly by certain polyphosphate accumulating organisms (PAOs) called *Candidatus* Accumulibacter. Recently, actinobacteria belonging to the genus *Tetrasphaera* have been identified as putative polyphosphate accumulating organisms. New investigations show that they comprise a larger fraction of the biomass in full-scale Danish wastewater treatment plants than *Candidatus* Accumulibacter (8-12% and 3-7%, respectively) and may therefore be more important in the phosphorus removal process. Little is known about the diversity and physiology of *Tetrasphaera* but preliminary results indicate that their ecological niche is different from *Candidatus* Accumulibacter.

AIM

- Determine the diversity and abundance of *Tetrasphaera* in Danish treatment plants with enhanced biological phosphorus removal (EBPR).
- Develop a metabolic model for phosphate-removing *Tetrasphaera* based on genomic investigations and pure culture studies.

METHODS

Identification and quantification

Quantitative FISH using published and novel species-specific probes

Development of metabolic model

- Paired-end genome sequencing of four *Tetrasphaera* isolates using the Illumina platform.
- Annotation using MaGe (Microbial Genome Annotation Platform) from MicroScope.
- Validating the genomic findings with pure culture studies.

CONCLUSIONS

- **A high abundance and diversity of *Tetrasphaera* were identified in Danish full-scale EBPR plants.**
- ***Tetrasphaera* are potentially novel and ecologically relevant polyphosphate accumulating organisms in full-scale EBPR plants with a different physiology in EBPR plants compared to *Candidatus* Accumulibacter.**

Identification and quantification

Fig. 1: Phylogenetic tree of 16S rRNA gene sequences of *Tetrasphaera* from EBPR plants. The sequences group in 3 clusters. 2 clusters include isolated *Tetrasphaera*.

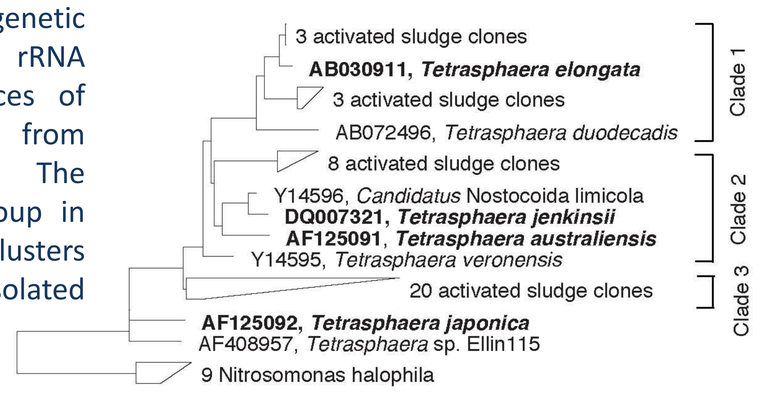
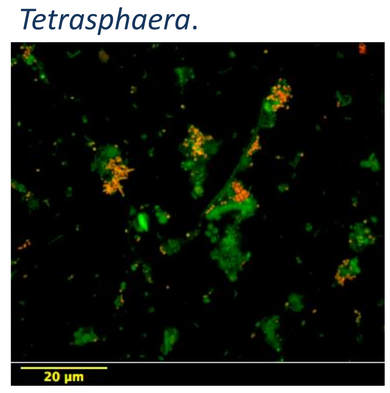
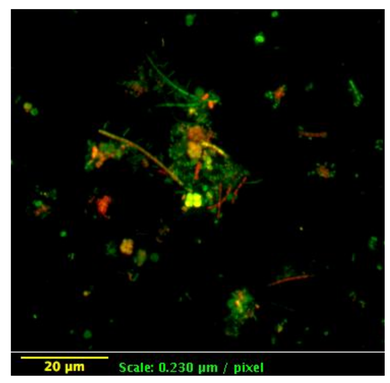


Fig. 2: FISH images of *Tetrasphaera*.





Probe	Cluster	Treatment Plant (% EUBmix ± SD)				
		Ejby Mølle	Hjørring	Åbenrå	Skive	Odense
Elo1250	1	0	0	< 1	3.2± 1.3	1.2 ± 1.0
Tet823	1	< 1	3.4 ±1.6	0	0	0
Tet266	1	5.5 ± 2.0	4.2±1.2	4.1±1.4	< 1	4.3 ± 1.0
Tet842	2	< 1	1.1 ± 1.0	3 ± 1.3	< 1	< 1
Tet831	2	0	0	3.1 ±1.5	0	0
Tet892	2	5.4 ± 1.3	4.4 ± 1.0	4.5 ± 1.5	4.3 ± 1.0	4.4 ± 1.0
Tet87	2	2.3 ± 1.7	1.2 ± 1	< 1	< 1	< 1
Tet174	2	5.2 ± 1.3	4 ± 1.0	2.9 ± 1.0	1.7 ± 1.0	4.5 ± 1.2
Tet654	3	6.8 ± 2.0	5.7 ± 1.1	5.5 ± 1.8	5.4 ± 2.0	5.2 ± 1.4
Tet19	3	4.3 ± 1.5	4.1 ± 1.0	3.2 ± 1.0	3.5 ± 1.0	4.3 ± 1.0
PAO mix (*Accumulibacter*)		5.4 ± 1.0	3.1 ± 1.3	4.8 ± 1.5	4.5 ± 1.0	5.4 ± 1.2

Table 1: Abundance of *Tetrasphaera* in Danish EBPR plants determined using qFISH.

Development of metabolic model

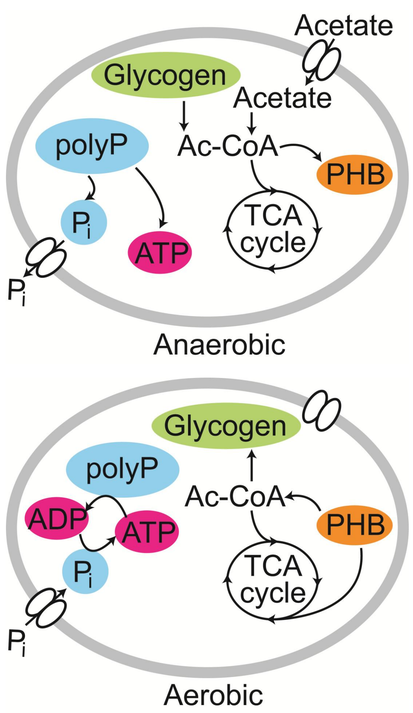


Fig. 3: Simplified metabolic model of *Candidatus* Accumulibacter during the EBPR process (Martín et al., 2006).

Pathway	*Accumulibacter*	*T. australiensis*	*T. elongata*	*T. jenkinsii*	*T. japonica*
Glycolysis	+	+	+	+	+
Gluconeogenesis	+	+	+	+	+
Glycogen synthesis	+	+	+	+	+
Glycogenolysis	+	+	+	+	+
TCA cycle	+	+	+	+	+
Glyoxylate shunt	+	-	-	-	-
PHA synthesis	+	-	-	-	+
Polyphosphate metabolism	+	+	+	+	+
Fermentation	-	+	+	+	+
Acetate uptake	+	+	+	+	+
Glucose uptake	-	+	+	+	+
Glutamate/aspartate uptake	-	+	+	+	+

Table 2: Potential metabolic pathways identified by genomic investigations.
+: present
-: not present

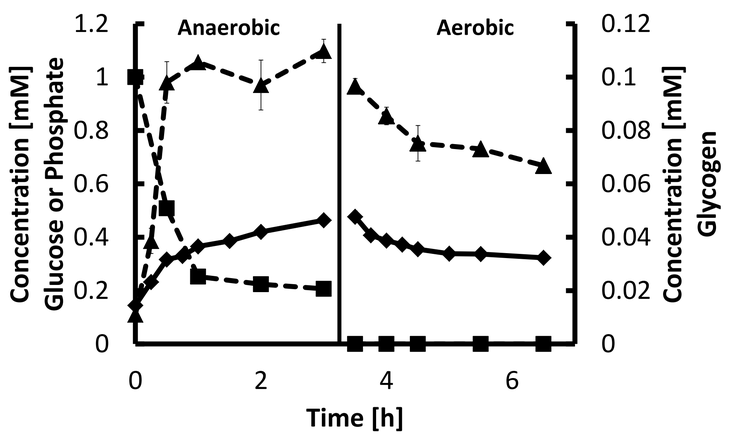


Fig. 4: The ability of *T. elongata* to release Pi (triangles) and produce glycogen (diamonds) in the anaerobic phase feed with glucose (squares) (0-3 h) and take up Pi and consume glycogen in the aerobic phase (3.5-6.5 h).

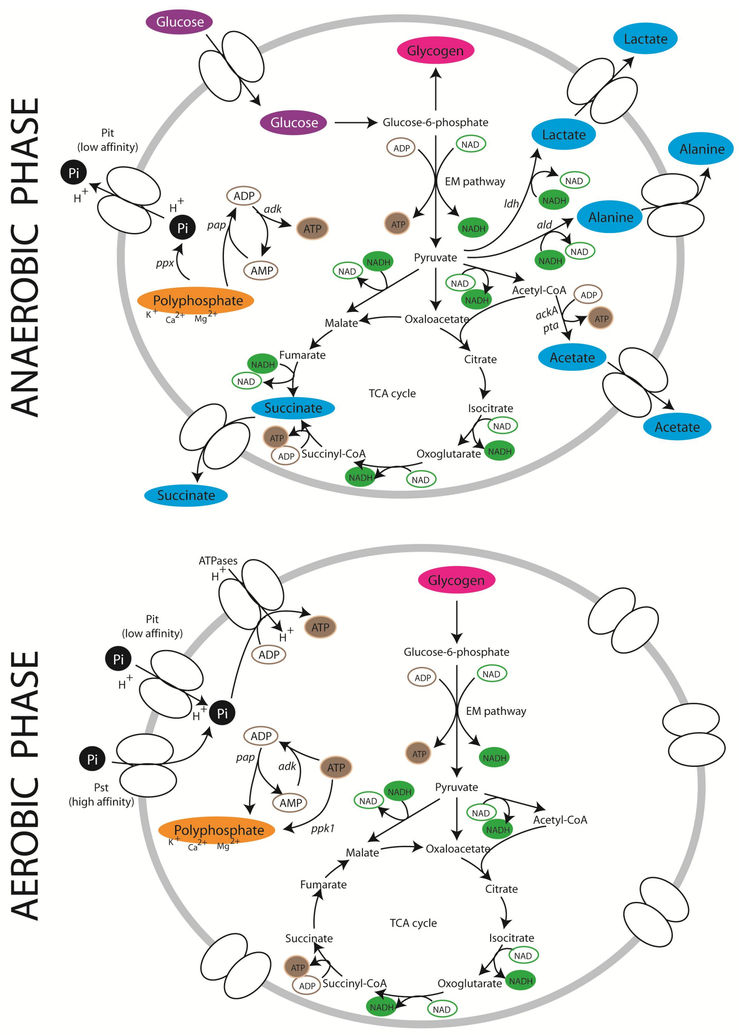


Fig. 5: Metabolic model for *Tetrasphaera*. The key metabolic features enabling *Tetrasphaera* to compete in full-scale EBPR plants are shown.